

Express Mail No.: EV504788940US

Atty. Dkt. No. 15060-42

REMARKS

Please enter the foregoing preliminary amendment prior to examination of the present application. Applicant respectfully submits that this Amendment presents no new matter. Early passage to issue is requested.

Respectfully submitted,



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IN THE DRAWINGS

Applicants respectfully request approval of the following drawing changes.

Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33 are being amended to remove excessive text. Applicants hereby submit an "Annotated Copy" of Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33 showing the requested changes in red permanent ink, and a "Replacement Sheet" incorporating the changes to Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33.

No new matter has been added.

FIGURE 2

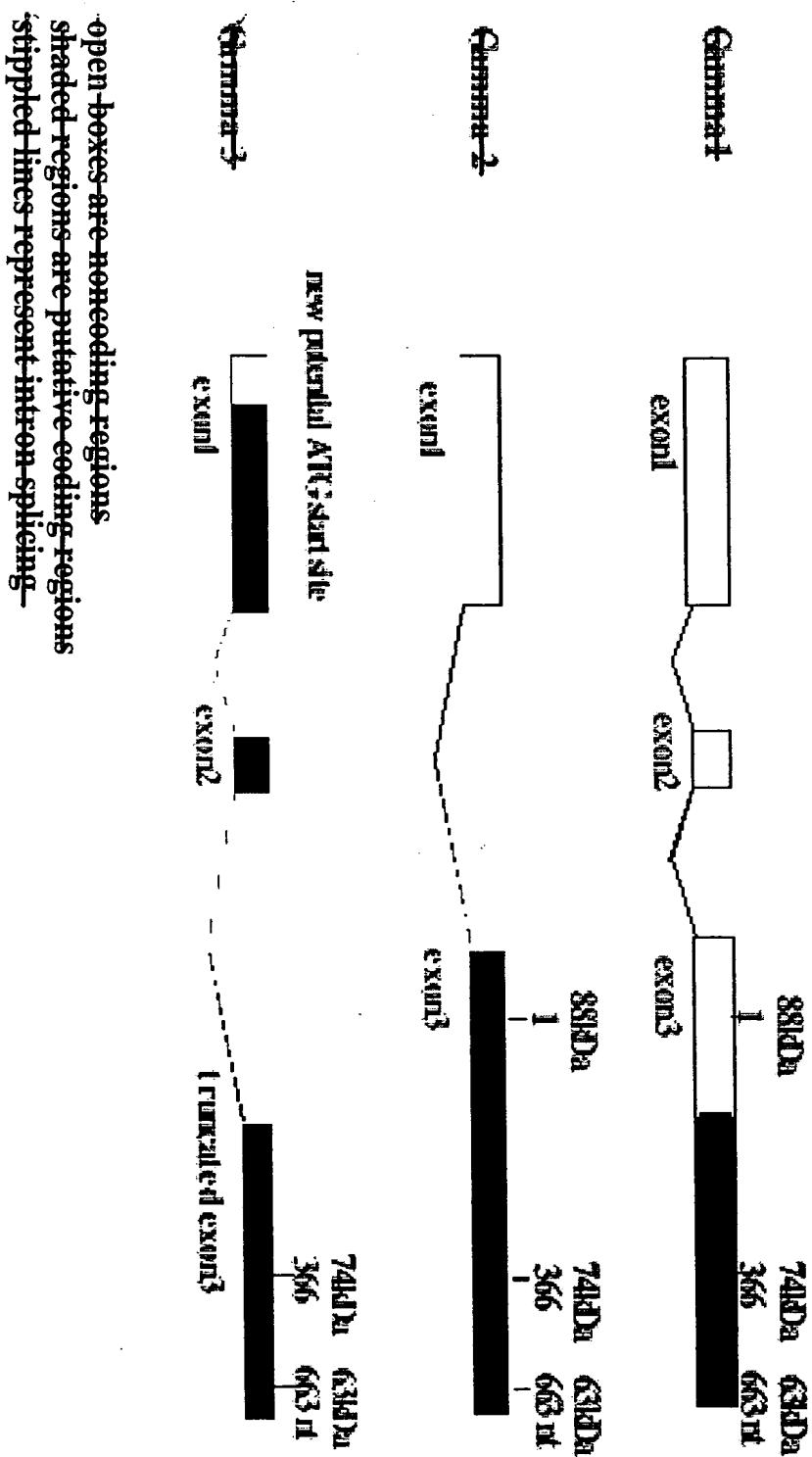
iPLA₂γ Splice Variants

5/61

Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR
Inventor: Richard W. Gross et al.
Docket No.: 15060-42
Gordon F. Sieckmann, Phone 314-621-5070



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open boxes are non-coding regions
shaded regions are putative coding regions
stippled lines represent intron-splicing

FIGURE 10

Potential Alternative-Exon-5-Splice-Variant of Human-iPLA₂^γ

A. Reported Splice Sequence (gc/ag)

<u>Exon 5 (SEQ ID NOS 43-44)</u>	<u>Intron 5</u>	<u>Exon 6 (SEQ ID NOS 45-46)</u>	<u>Source</u>
...CAG CGA GAA AAG	gcaagg...tttgttag	ATT ATC GCA AGG GTG AGT	(Tanaka et al)
Q R E K		I I A R V S	BBRE 272, 320, 2000

B. Potential Splice Variant (gt/agt)

<u>Exon 5 (SEQ ID NOS 47-48)</u>	<u>Intron 5</u>	<u>Exon 6</u>	<u>Source</u>
..GAA AAG GCA AGT TGT TCA GT	gttgtt..tcgcaag	G V	(Gross-lab)
E K A S C S V		TGT AGT S	BBRE 275, 9937, 2000

The incidence of gt/ag splice variants like the one shown in "A" is 0.56%. The variant "A" has been reported in the literature, reported in GenBank, and cloned in our lab.

The splice variant gt/tg occurs with a frequency of 08.71% among genes. However, variant "B" iPLA₂^γ sequence has not been cloned.

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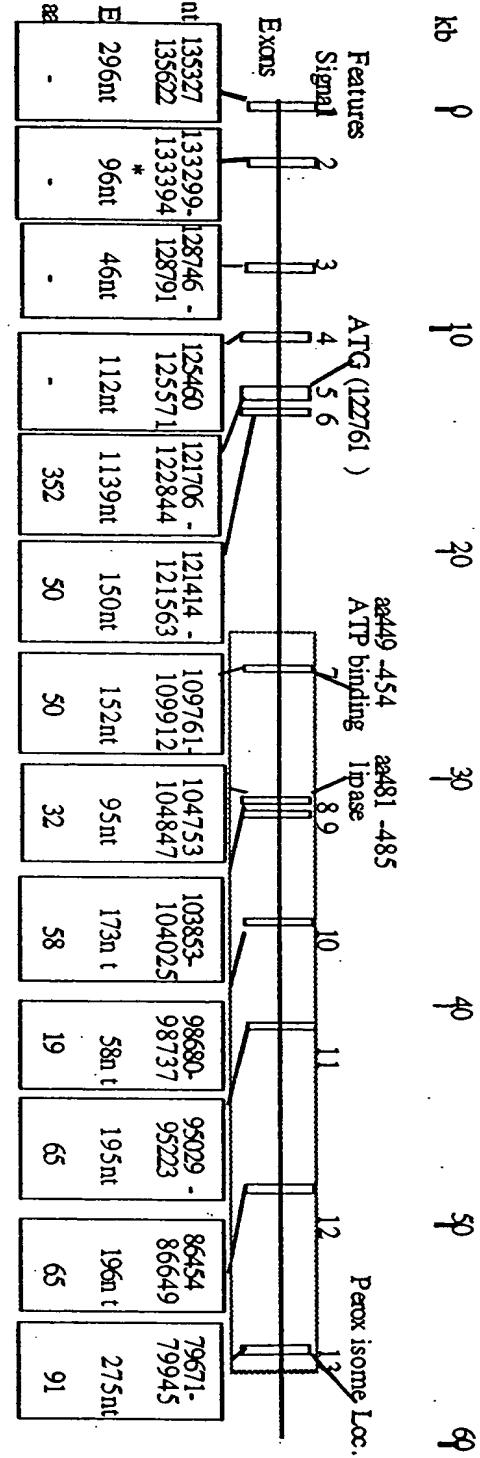
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24/61

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FIGURE 13



*5' end has been also
 been reported as 13314
 and 133464 in GenBank

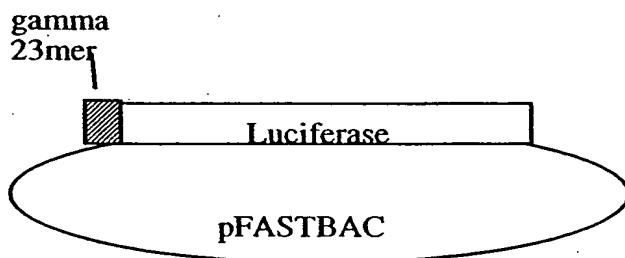
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32/61

ANNOTATED MARKED UP

FIGURE 21

Additionally, iPLA₂ γ sequences were inserted by ligation of 15-23mer annealed phosphorylated oligonucleotide pairs 5' of full length luciferase coding sequence cloned into pFASTBAC via NdeI/XbaI restrictions and then luciferase activity of recombinant protein produced in the Sf9 system was subsequently measured using the Luciferase Assay System of Promega.



34 / 61

FIGURE 23. iPLA₂-Repressor-Region

Phosphorylated oligo-pairs
for repressor of iPLA₂ in the interferase expression system:

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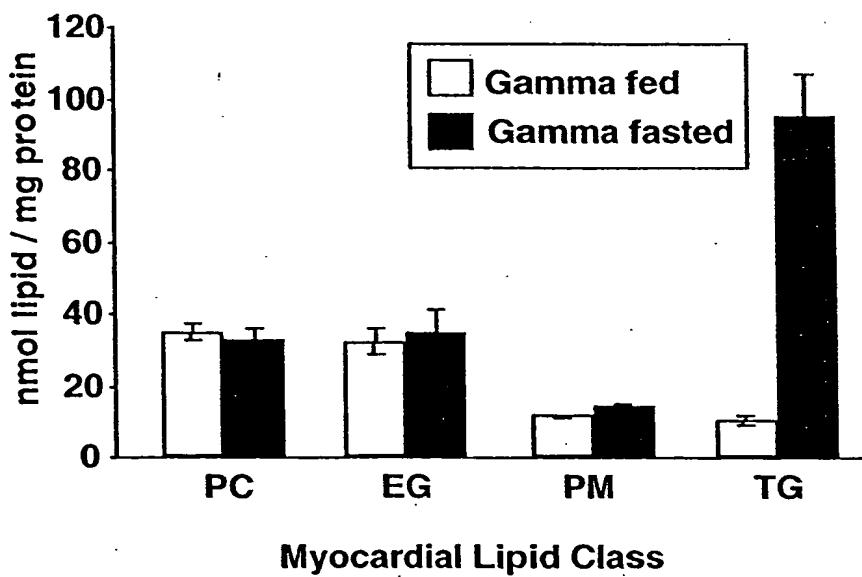
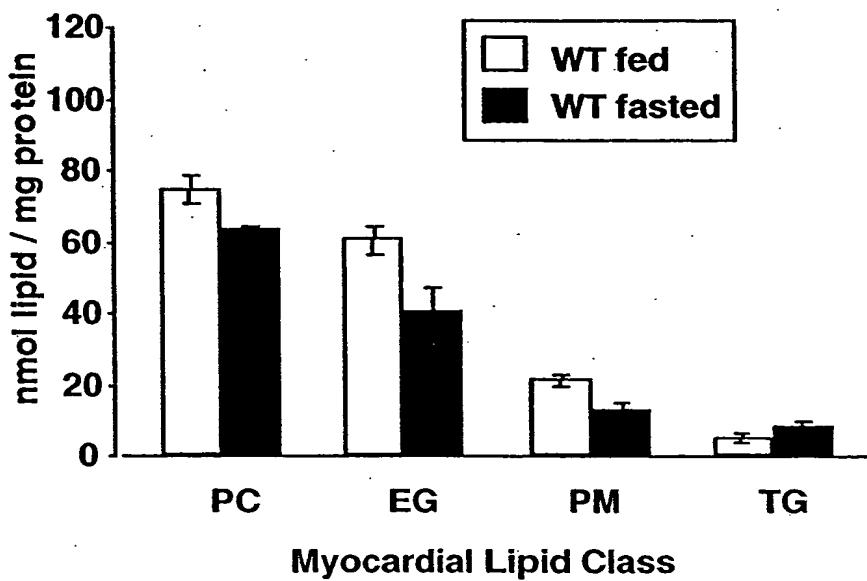
SEQ ID NO: 10 atgatttcacgttagctcaatttaagccaaatcccattttaaaggatcgatagtggctggtaaacaaaaacatcaaaca
SEQ ID NO: 32 tcgacccgttgatttcacgtttagctcaatt
SEQ ID NO: 36 ggactaaagtgcataatcgaggttaaccgg
SEQ ID NO: 33 tcgactaaggccaaatccaaattttaa
SEQ ID NO: 37 gattcgggtcaagggtttaaaatccgg
SEQ ID NO: 34 tcgacgaaagtatcgatagtggtgg
SEQ ID NO: 38 gctttcatagcctatccacggaccgg
SEQ ID NO: 35 tcgacttaaaacagaaaaaacatcaaaca
SEQ ID NO: 39 gaattttgtctttttagtttgtccgg

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ANNOTATED MARKED UP

37/61

Fig. 26 Myocardial TAG Content of Fasted WT vs iPLA₂ γ Transgenic Mice



PC = Phosphatidylecholine

EG = Ethanolamine Glycerophospholipids

PM = Plasmalogen

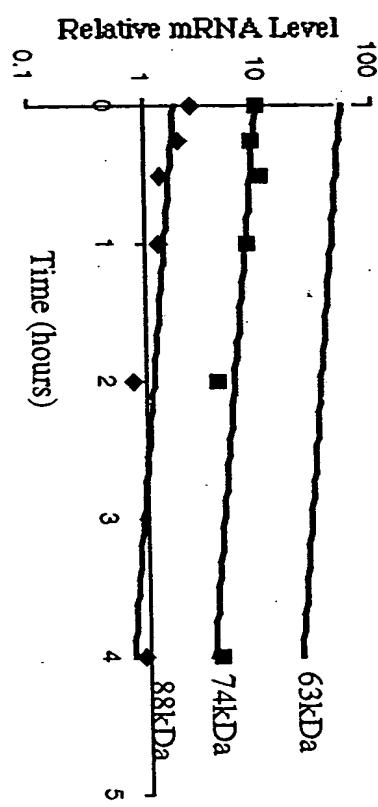
TG = Triacylglyceride

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ANNOTATED MARKED UP

39 / 61

FIGURE 28. Quantitative PCR analysis of mRNA stability of treated iPLA₂ γ -S90 Expression



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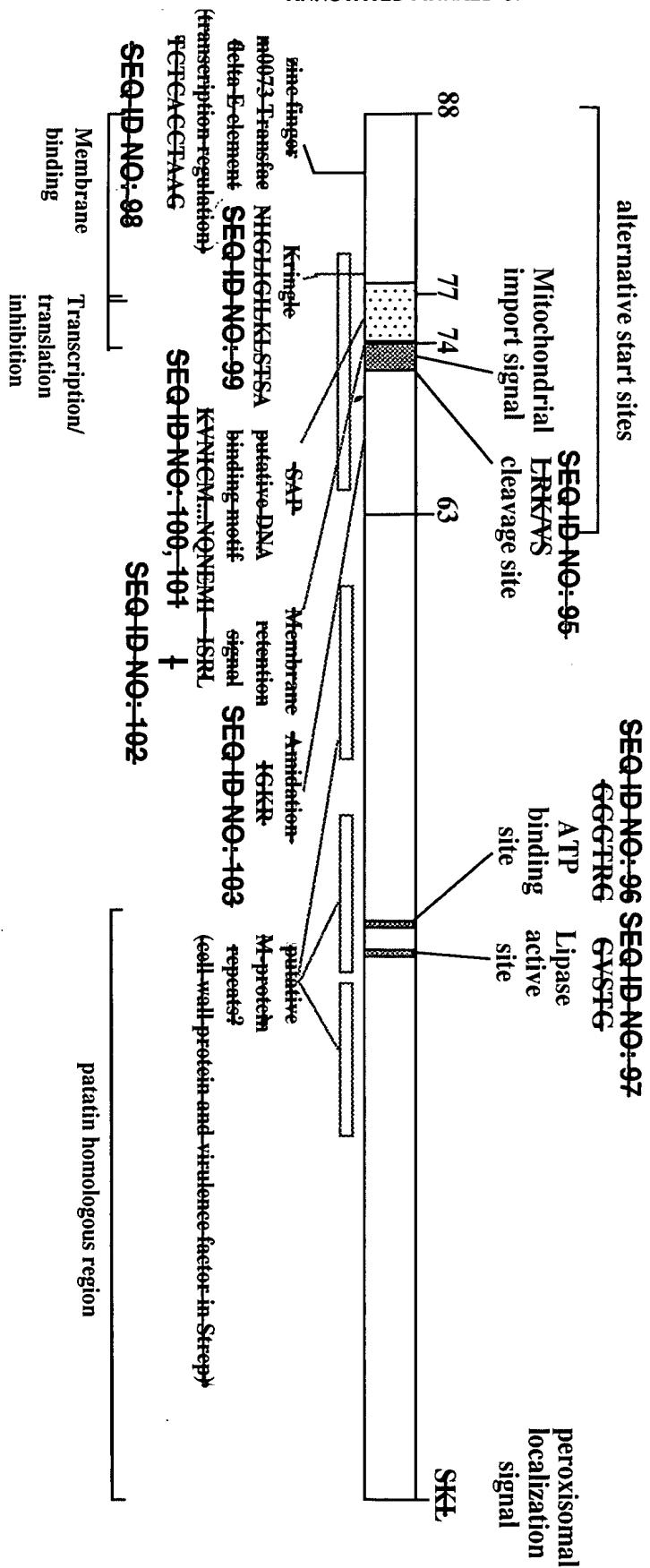
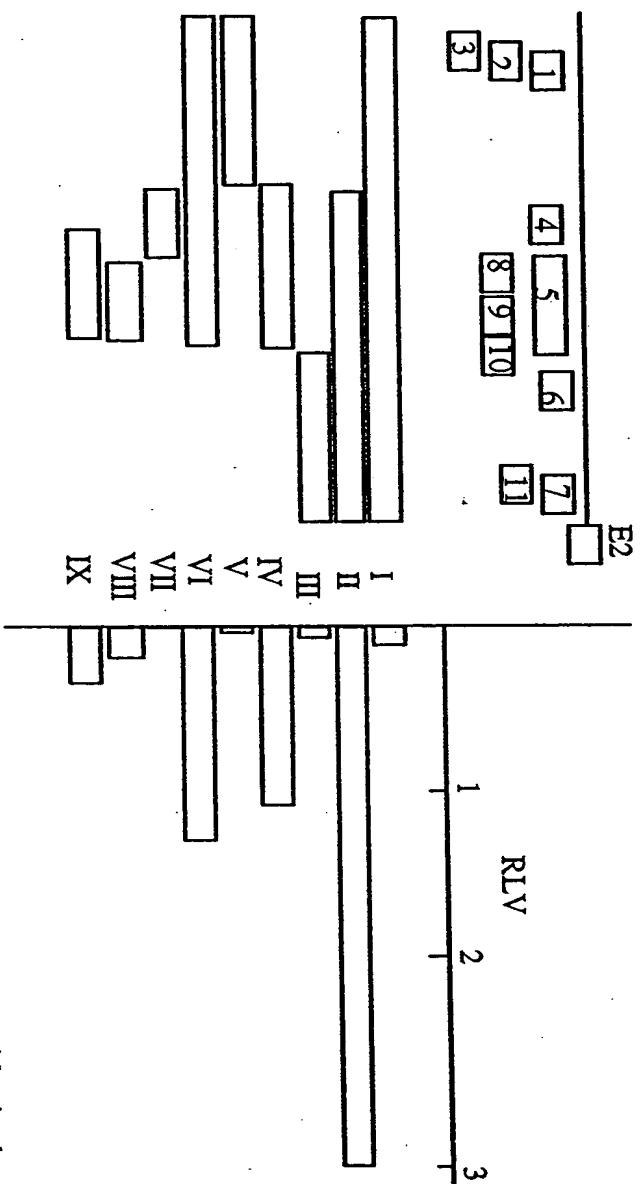


FIGURE 31

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FIGURE 33

Promoter Analysis of iPLA₂^γ Pre-exon-2



Conclusion: sequence upstream of exon-2 has promoter activity. Enhancer activity resides in the region 200-400nt upstream of exon-2 (fragment IV). This region contains a CACG VNTR like sequence as well as sequences that match consensus sites for Sp1 (8), GATA1 (9), p300 (4), and CenH (10). GC regions upstream (1) and downstream (7) of this positive promoter region commonly are negative regulatory elements. Truncated fragments (II and VI) each lacking a GC region have minimal promoter activity while fragments (III and V) containing the GC regions but lacking region IV have maximal promoter activity. Presumably both GC regions are required for maximal inhibition. Region IV may have less than optimal promoter activity if positive promoter elements are immediately upstream or downstream of region IV.